

DATA EVALUATION RECORD
ACUTE EC₅₀ TEST WITH AN ESTUARINE/MARINE MOLLUSK
SHELL DEPOSITION STUDY
72-3(B), OPPTS 850.1025

1. **CHEMICAL:** 2,4-D DMA, aminopyralid **PC Code No.:** 030019 and 005100/005029

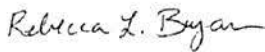
2. **TEST MATERIAL:** GF-2633 **Purity:** 43.0% 2,4-D DMA + 8.43%
aminopyralid

3. **CITATION**

Authors: Gerke, A.
Title: GF-2633: Effect on New Shell Growth of the Eastern Oyster
(*Crassostrea virginica*)
Study Completion Date: October 27, 2011
Laboratory: ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, MO 65202
Sponsor: Dow AgroSciences LLC
9330 Zionsville Rd.
Indianapolis, IN 46268
Laboratory Report ID: 66957
MRID No.: 48939502
DP Barcode: 289122

4. **REVIEWED BY:**

Rebecca L. Bryan
Staff Scientist, CSS-Dynamac

Signature: 
Date: 12/18/15

APPROVED BY:

John Marton, Ph.D.
Environmental Scientist, CDM Smith, Inc.


Signature: 
Date: 5/23/16

5. **APPROVED BY:**

Primary Reviewer:
Rebecca Lazarus, Ph.D.
EPA/OPP/EFED/ERB1

Signature: 
Date: 6/3/16

Secondary Reviewer:
{.....}
OPP/EFED/ERB

Signature: 
Date: 6/3/16

6. **DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to shell deposition in oysters. It is not intended to prescribe conditions to any external party for conducting this study or to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study

7. **STUDY PARAMETERS:**

Test Species: Eastern Oyster (*Crassostrea virginica*)
Age or Size of Test Organism: Mean valve height: 33 mm (range: 30-38 mm)
Definitive Test Duration: 96 hours
Study Method: Flow-through
Type of Concentrations: Mean measured

8. **CONCLUSIONS:**

Results Synopsis:

<u>GF-2633</u>	
IC ₅₀ : 94.1 mg form/L	95% C.I.: 82.6-107 mg form/L
<u>2,4-D DMA</u>	
IC ₅₀ : 40.4 mg ai/L	95% C.I.: 35.5-46.1 mg ai/L
<u>Aminopyralid</u>	
IC ₅₀ : 7.93 mg ai/L	95% C.I.: 6.96-9.05 mg ai/L

9. **ADEQUACY OF THE STUDY:**

This study is **scientifically sound** and is classified as **acceptable**.

10. **BACKGROUND:**

11. GUIDELINE DEVIATIONS:

This study followed a protocol that was based on the procedures outlined in the US EPA Series 850-Ecological Effects Test Guidelines, OPPTS Number 850.1025: Oyster Acute Toxicity Test (Shell Deposition).

The following deviation from the OPPTS 850.1025 guidelines was noted:

1. The total organic carbon of the dilution water was not reported.

This deviation does not affect the validity of the study.

12. SUBMISSION PURPOSE:

This study was submitted to provide information on the effects of GF-2633 on Eastern oyster (*Crassostrea virginica*) shell deposition following acute exposure for the purpose of registration review.

13. MATERIALS AND METHODS:**A. Test Organisms**

Guideline Criteria	Reported Information
<u>Species</u> Preferred species are the Pacific oyster (<i>Crassostrea gigas</i>) and the Eastern oyster (<i>Crassostrea virginica</i>)	Eastern oyster (<i>Crassostrea virginica</i>)
<u>Mean valve height</u> 25 - 50 mm along the long axis	33 ± 1.9 mm (range: 30-38 mm)
<u>Supplier</u>	Circle C Oyster Ranchers Association (Ridge, Maryland)
Are all oysters from same source?	Yes
Are all oysters from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 10 days	2 days (acceptable, as per correspondence with EPA)
Wild caught organisms were quarantined for 7 days?	NA
Were there signs of disease or injury?	None reported
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	NA
<u>Amount of peripheral shell growth removed prior to testing</u>	Recently deposited shell (3 to 5 mm) was removed from all oysters with an electric grinder.
<u>Feeding during the acclimation</u> Must be fed to avoid stress.	Oysters were provided with a suspension of marine microalgae (Instant Algae Shellfish Diet 1800) from Reed MariCulture, Inc. (Campbell, CA), at least once per day during acclimation.
<u>Pretest Mortality</u> <3% mortality 48 hours prior to testing	None

C. Test System

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Natural unfiltered seawater from an uncontaminated source.	Laboratory saltwater prepared by adding Crystal Sea Marinemix (Marine Enterprises International, Inc., Baltimore, MD) to laboratory freshwater at a target salinity of 20±2‰. The laboratory freshwater consisted

Guideline Criteria	Reported Information
	<p>of well water blended with well water demineralized by reverse osmosis to yield water with a total hardness ranging from 130 to 160 mg CaCO₃/L. Prior to use, the dilution water was filtered (1 µm) and UV sterilized.</p> <p>The results of the most recent water analysis (February 2011) for selected chemical parameters and potential contaminants was provided.</p>
Does water support test animals without observable signs of stress?	Yes
<u>Salinity</u> 30-34‰ (parts per thousand) salinity, weekly range <6‰	19.6 to 19.8‰
<u>Water Temperature</u> 15-30°C, consistent in all test vessels	19.4 to 20.8°C
<u>pH</u>	7.6 to 8.2
<u>Dissolved Oxygen</u> >60% throughout	4.9 to 7.8 mg/L (62 to 99% saturation).
<u>Total Organic Carbon</u>	Not reported
<u>Test Aquaria</u> Should be constructed of glass or stainless steel.	Glass aquaria (22 cm x 37 cm x 18 cm), filled with <i>ca.</i> 8.4 L test solution at a depth of 11 cm.
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	A proportional diluter was used to deliver the test substance stock solutions into the test chambers.
<u>Flow rate</u> Consistent flow rate	<i>Ca.</i> 8.1 volume additions/24 hours

Guideline Criteria	Reported Information
Was the loading of organism such that each individual sits on the bottom with water flowing freely around it?	Yes
<u>Photoperiod</u> 16 hours light, 8 hours dark	16 hours light, 8 hours dark; with 30-minute transition periods. Light intensity was 610 lux on Day 0.
<u>Solvents</u> Not to exceed 0.5 ml/L	NA

D. Test Design

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If EC ₅₀ >100 mg/L with 30 or more oysters, then no definitive test is required.	A range-finding test was conducted July 28 to August 1, 2011 at nominal concentrations of 0 (control), 13, 22, 36, 60, and 100 mg ai/L. After 96 hours, the percent shell reduction ranged from 12% in 22 mg ai/L group to 59% in the 100 mg ai/L group.
<u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series	<u>GF-2633</u> 0 (negative control), 13, 22, 36, 60, and 100 mg GF-2633/L <u>Aminopyralid triisopropanolammonium</u> 1.10, 1.85, 3.03, 5.06, and 8.43 mg ai/L <u>2,4-D dimethylamonium</u> 5.59, 9.46, 15.5, 25.8, and 43.0 mg ai/L
<u>Number of Test Organisms</u> Minimum 20 individual per test level and in each control	20/level, equally divided between 2 replicates

Guideline Criteria	Reported Information
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
<u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured hourly in at least one chamber 2. <u>DO and pH</u> Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	1) Temperature was measured in each test chamber daily, as well as continuously in Replicate A of the control. 2) The DO and pH were measured in each test chamber daily.
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the test? (Optional)	Yes, using HPLC-UV at 0 and 96 hours. Samples were analyzed for aminopyralid and corrected based on purity to represent measured concentration based on the formulated product.

14. **REPORTED RESULTS:**

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. Signed and dated Data Confidentiality, Quality Assurance, and GLP compliance statements were provided. The study was conducted in accordance with US EPA GLP standards (40 CFR part 160), with one exception: the most recent analyses of saltwater for potential contaminants (Feb. 2011) was not performed according to the stated GLP.

Guideline Criteria	Reported Information
<u>Control Mortality</u> Not more than 10% of control organisms may die or show abnormal behavior.	0%
<u>Control Shell Deposition</u> Must be at least 2 mm.	3.86±0.57 mm
<u>Recovery of Chemical</u>	95-101% of nominal The mean measured concentrations were 13.0, 20.9, 36.1, 60.3, and 98.2 mg GF-2633/L. <u>Aminopyralid triisopropanolammonium</u> 1.10, 1.76, 3.04, 5.08, and 8.28 mg ai/L <u>2,4-D dimethylamonium</u> 5.59, 8.99, 15.5, 25.9, and 42.2 mg ai/L
Raw data included?	Yes
Signs of toxicity (if any) were described?	No signs of toxicity were observed.

Mortality and Shell Growth^a

Concentration (mg GF-2633/L)		Number Per Level	Number Dead (% mortality)	Mean Shell Deposition (mm ± SD)	Mean Percent Reduction
Nominal	Mean-measured				
Neg. Control	<MQL ^b	20	0	3.86±0.57	NA
13	13.0	20	0	3.95±0.06	-2
22	20.9	20	0	3.57±0.41	8
36	36.1	20	0	3.86±0.59	0
60	60.3	20	0	2.82±0.04	27
100	98.2	20	0	1.85±0.09	52*

a Data were obtained from Table 2 on page 22 of the study report.

b MQL = 1.90 mg ai/L

* Statistically significant reduction as compared to the control (p=0.05).

B. Statistical Results

Statistical Method: Statistical analyses were performed using SAS[®] (version 9.1) statistical software. Prior to a one-tailed Dunnett's test, the shell deposition data were evaluated homogeneity of variance using a Bartlett's test. All data met the assumptions of normality and homogeneity of variance and a parametric analysis was performed. The EC₅₀ was estimated using a four-parameter logistic (sigmoid-shaped) model.

96 hour IC₅₀ based on nominal concentrations:

IC₅₀: 96 mg ai/L

95% C.I.: 88-100 mg ai/L

NOAEC: 60 mg ai/L

15. VERIFICATION OF STATISTICAL RESULTS:

Parameter	IC ₅₀ (95% C.I.)
GF-2633*	94.1 (82.6-107) mg form/L
2,4-D dimethylammonium	40.4 (35.5-46.1) mg ai/L
Aminopyralid triisopropanolammonium	7.93 (6.96-9.05) mg ai/L
Software	CETIS, Version 1.8.7.12
Backend settings	10/20/15

*GF-2633 is an adjusted mixture of the proportions of two active ingredients 2,4-D DMA and aminopyralid at the onset of the study.

16. REVIEWER'S COMMENTS:

The reviewer's results were more conservative than those reported by the study author and were expressed as the formulated product, as well as the individual active ingredients. Therefore, the reviewer's results are reported in the Conclusions section of this DER.

After 96 hours, the percent shell reduction was -2, 8, 0, 27, and 52% in the nominal 13, 22, 36, 60, and 100 mg ai/L groups compared to the control. The shell growth was significantly reduced in the 100 mg ai/L group. The EC₅₀ (95% C.I.) was estimated as 96 mg ai/L and the NOAEC was 60 mg ai/L.

GF-2633 is an adjusted mixture based on proportions of 2,4-D DMA and aminopyralid at the onset of the study. The formulated product, GF-2633, consisted of 8.43% wt/wt aminopyralid triisopropanolammonium (i.e, 4.38% wt/wt aminopyralid acid equivalent) and 43.0% wt/wt 2,4-D dimethylammonium (35.7% wt/wt 2,4-D acid equivalent). When calculating the nominal and mean-measured concentrations, the reviewer used the active ingredients (not the acid equivalents).

17. CONCLUSIONS: This study is scientifically sound and is classified as acceptable. Based on the results of this study, GF-2633 and DMA would be classified as **slightly toxic** and aminopyralid would be classified as **moderately toxic**.

96 hour LC₅₀:

GF-2633

IC₅₀: 94.1 mg form/L 95% C.I.: 82.6-107 mg form/L

NOAEC: 60.3 mg GD-2633/L

LOAEC: 98.2 mg GF-2633/L (based on mortality)

2,4-D DMA

IC₅₀: 40.4 mg ai/L 95% C.I.: 35.5-46.1 mg ai/L

NOAEC: 25.9 mg 2,4-D DMA/L

LOAEC: 42.2 mg 2,4-D DMA/L (based on mortality)

Aminopyralid

IC₅₀: 7.93 mg ai/L 95% C.I.: 6.96-9.05 mg ai/L

NOAEC: 5.08 aminopyralid/L

LOAEC: 8.28 aminopyralid/L (based on mortality)

18. REFERENCES:

Gerke, A. (2011). GF-2633: "Effect on New Shell Growth of the Eastern Oyster (*Crassostrea virginica*)", DAS, ABC Study No. 66957. MRID: 48939502.

All other references were standard guidelines or methodologies.

CETIS Summary Report

Report Date: 06 Jan-16 08:58 (p 1 of 1)
 Test Code: 48939502 24D-DM | 09-5931-3513

OPPTS 850.1025 Acute Invert (Oyster)

ABC Labs

Batch ID:	21-3573-7359	Test Type:	Oyster (96-h)	Analyst:	
Start Date:	25 Aug-11	Protocol:	OPPTS 850.1025 Acute Invert (Oyster)	Diluent:	Laboratory Water
Ending Date:	06 Jan-16 08:54	Species:	Crassostrea virginica	Brine:	Crystal Sea
Duration:	1595d 9h	Source:	Circle C Oysters, Ridge, MD	Age:	
Sample ID:	08-9873-9468	Code:	48939502 24D-DM	Client:	CDM Smith - J. Marton
Sample Date:	25 Aug-11	Material:	2,4-D, dimethylamine salt	Project:	Herbicide
Receive Date:	06 Jan-16 08:54	Source:	Dow AgroSciences		
Sample Age:	NA	Station:			

Batch Note: PC Code 030019+005100, MRID 48939502, mean-measured 2,4-D, DMA concentrations

Sample Note: PC Code 030019+005100, MRID 48939502, mean-measured 2,4-D, DMA concentrations

Point Estimate Summary

Analysis ID	Endpoint	Level	mg ai/L	95% LCL	95% UCL	TU	Method
03-3661-0718	Shell Deposition	IC5	14.5	N/A	19.6		Nonlinear Regression
		IC10	18.2	9.29	23.4		
		IC15	21.2	14.6	26.5		
		IC20	23.9	18.3	29.1		
		IC25	26.6	21.6	31.5		
		IC40	34.5	30.5	38.9		
		IC50	40.4	35.5	46.1		

Shell Deposition Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	2	3.86	-1.22	8.94	3.46	4.26	0.4	0.566	14.7%	0.0%
5.59		2	3.96	3.38	4.53	3.91	4	0.045	0.0636	1.61%	-2.46%
8.99		2	3.57	-0.115	7.25	3.28	3.86	0.29	0.41	11.5%	7.51%
15.5		2	3.87	-1.41	9.14	3.45	4.28	0.415	0.587	15.2%	-0.13%
25.9		2	2.82	2.44	3.2	2.79	2.85	0.03	0.0424	1.5%	26.9%
42.2		2	1.85	1.09	2.61	1.79	1.91	0.06	0.0849	4.59%	52.1%

Shell Deposition Detail

C-mg ai/L	Control Type	Rep 1	Rep 2
0	Negative Control	3.46	4.26
5.59		4	3.91
8.99		3.28	3.86
15.5		4.28	3.45
25.9		2.79	2.85
42.2		1.91	1.79

CETIS Summary Report

Report Date: 06 Jan-16 09:02 (p 1 of 1)
 Test Code: 48939502 amino | 05-0345-7366

OPPTS 850.1025 Acute Invert (Oyster)

ABC Labs

Batch ID:	00-6998-7885	Test Type:	Oyster (96-h)	Analyst:	
Start Date:	25 Aug-11	Protocol:	OPPTS 850.1025 Acute Invert (Oyster)	Diluent:	Laboratory Water
Ending Date:	06 Jan-16 08:58	Species:	Crassostrea virginica	Brine:	Crystal Sea
Duration:	1595d 9h	Source:	Circle C Oysters, Ridge, MD	Age:	
Sample ID:	09-2456-4736	Code:	48939502 amino	Client:	CDM Smith - J. Marton
Sample Date:	25 Aug-11	Material:	Aminopyralid	Project:	Herbicide
Receive Date:	06 Jan-16 08:58	Source:	Dow AgroSciences		
Sample Age:	NA	Station:			

Batch Note: PC Code 030019+005100, MRID 48939502, mean-measured aminopyralid concentrations

Sample Note: PC Code 030019+005100, MRID 48939502, mean-measured aminopyralid concentrations

Point Estimate Summary

Analysis ID	Endpoint	Level	mg ai/L	95% LCL	95% UCL	TU	Method
20-3562-2558	Shell Deposition	IC5	2.85	N/A	3.85		Nonlinear Regression
		IC10	3.57	1.82	4.6		
		IC15	4.16	2.87	5.2		
		IC20	4.7	3.59	5.71		
		IC25	5.21	4.23	6.18		
		IC40	6.78	5.98	7.63		
		IC50	7.93	6.96	9.05		

Shell Deposition Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	2	3.86	-1.22	8.94	3.46	4.26	0.4	0.566	14.7%	0.0%
1.1		2	3.96	3.38	4.53	3.91	4	0.045	0.0636	1.61%	-2.46%
1.76		2	3.57	-0.115	7.25	3.28	3.86	0.29	0.41	11.5%	7.51%
3.04		2	3.87	-1.41	9.14	3.45	4.28	0.415	0.587	15.2%	-0.13%
5.08		2	2.82	2.44	3.2	2.79	2.85	0.03	0.0424	1.5%	26.9%
8.28		2	1.85	1.09	2.61	1.79	1.91	0.06	0.0849	4.59%	52.1%

Shell Deposition Detail

C-mg ai/L	Control Type	Rep 1	Rep 2
0	Negative Control	3.46	4.26
1.1		4	3.91
1.76		3.28	3.86
3.04		4.28	3.45
5.08		2.79	2.85
8.28		1.91	1.79

CETIS Summary Report

Report Date: 06 Jan-16 08:53 (p 1 of 1)
 Test Code: 48939502 form | 08-0407-0504

OPPTS 850.1025 Acute Invert (Oyster)

ABC Labs

Batch ID: 21-0276-6281	Test Type: Oyster (96-h)	Analyst:
Start Date: 25 Aug-11	Protocol: OPPTS 850.1025 Acute Invert (Oyster)	Diluent: Laboratory Water
Ending Date:	Species: Crassostrea virginica	Brine: Crystal Sea
Duration: NA	Source: Circle C Oysters, Ridge, MD	Age: 33mm

Sample ID: 20-7528-7840	Code: 48939502 form	Client: CDM Smith - J. Marton
Sample Date: 25 Aug-11	Material: 2,4-D DMA + Aminopyralid	Project: Herbicide
Receive Date:	Source: Dow AgroSciences	
Sample Age: NA	Station:	

Batch Note: PC Code 030019+005100, MRID 48939502, mean-measured formulation concentrations

Sample Note: PC Code 030019+005100, MRID 48939502, mean-measured formulation concentrations

Point Estimate Summary

Analysis ID	Endpoint	Level	mg ai/L	95% LCL	95% UCL	TU	Method
18-2530-8836	Shell Deposition	IC5	33.8	N/A	45.7		Nonlinear Regression
		IC10	42.4	21.6	54.6		
		IC15	49.4	34.1	61.7		
		IC20	55.7	42.6	67.8		
		IC25	61.9	50.2	73.3		
		IC40	80.4	70.9	90.6		
		IC50	94.1	82.6	107		

Shell Deposition Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	2	3.86	-1.22	8.94	3.46	4.26	0.4	0.566	14.7%	0.0%
13		2	3.96	3.38	4.53	3.91	4	0.045	0.0636	1.61%	-2.46%
20.9		2	3.57	-0.115	7.25	3.28	3.86	0.29	0.41	11.5%	7.51%
36.1		2	3.87	-1.41	9.14	3.45	4.28	0.415	0.587	15.2%	-0.13%
60.3		2	2.82	2.44	3.2	2.79	2.85	0.03	0.0424	1.5%	26.9%
98.2		2	1.85	1.09	2.61	1.79	1.91	0.06	0.0849	4.59%	52.1%

Shell Deposition Detail

C-mg ai/L	Control Type	Rep 1	Rep 2
0	Negative Control	3.46	4.26
13		4	3.91
20.9		3.28	3.86
36.1		4.28	3.45
60.3		2.79	2.85
98.2		1.91	1.79